The Genomic Response of *Kochia scoparia* to Sublethal Doses of Glyphosate

Carly Claucherty[1], Eric L. Patterson[1], Todd A. Gaines[2]
[1]Michigan State University, [2]Colorado State University

Introduction

Glyphosate is the most used herbicide in the world and resistance is a costly threat to it's continued usefulness. A major mechanism for glyphosate resistance is increased copy number of the EPSPS gene, which has been demonstrated in at least nine species including *Kochia scoparia* (kochia). Interestingly, glyphosate is the only herbicide for which copy number variation (CNV) has been found to be a resistance mechanism. Abiotic stress has been shown to elicit genomic rearrangements; CNVs have been shown to form following heavy metal, drought, and salt stresses. These observations beg the question: does glyphosate elicit genomic responses like other abiotic stresses? Few studies have characterized the underlaying causes of genomic rearrangements in plants or the role that they play in gene expression, especially in non-model species.

Hypothesis: Glyphosate acts as an abiotic stress triggering signature changes in DNA methylation, up-regulation of stress-related genes, and allows for subsequent genomic rearrangements with a higher dose resulting in more drastic changes.

Objectives:

1. Perform RNA sequencing before and after application of sublethal doses of glyphosate to determine changes in gene expression
2. Identify differentially methylated loci before and after application of glyphosate through whole genome bisulfite sequencing (WGBS)
3. Intersect transcriptomic and epigenomic data to understand the relationship between genome wide methylation changes and gene expression changes

Materials and Methods

Plant material, glyphosate application, and sequencing: All leaf tissue was collected from a susceptible kochia line: '7710'. Plants were sprayed with either no glyphosate, a low sublethal dose (79 g a.e. ha^{-1}) or medium sublethal dose (158 g a.e. ha^{-1}) (rates were identified through a dose response assay). Tissue was taken from four individuals per treatment, pre- and three weeks post-herbicide application. DNA and RNA were extracted from both pre- and post- glyphosate tissue samples and sent for Illumina (NovaSeq) paired-end 150bp Whole Genome Bisulfite Sequencing (WGBS) and RNA-seq.

RNA sequencing analysis: Reads were filtered and trimmed using fastp and Salmon was used to align the reads to the kochia reference genome and quantify the transcripts. The R package, edgeR, was used to identify differentially expressed genes (DEGs).

WGBS sequencing analysis: Reads were filtered and trimmed using fastp. Bismark Bisulfite Mapper was used to align and call cytosine methylation sites across the genome and calculate the percentage of methylated reads. The R package, DSS, was used to identify differentially methylated loci (DMLs).

Results & Discussion

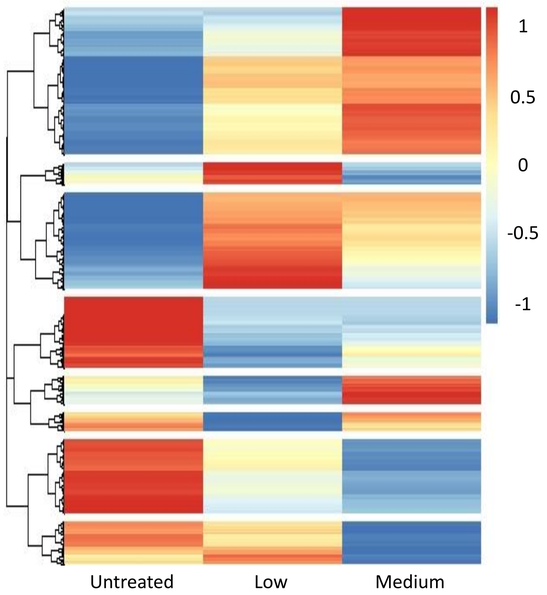

Figure 2. DEGs in all treatments and their increase or decrease in expression three weeks after glyphosate application relative to their initial expression with p-value (<0.01).

Experiment 1: Transcriptome Analysis

- Between pre and post timings, there were 5,457 DEGs in untreated, 2,151 DEGs in low-treated, and 1,436 DEGs in medium-treated (Fig. 1)
- DEGS in the untreated plants represent normal, background changes in gene expression over the three weeks of development.
- All differentially expressed genes were clustered into 8 different groups based on their overarching patten of expression across all treatments (Fig. 1)

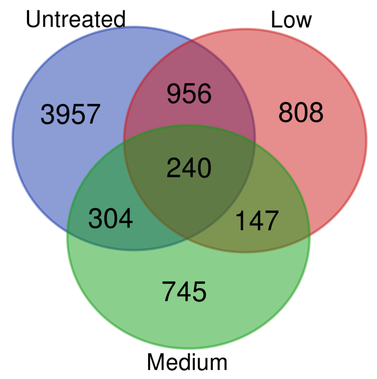

Figure 1. Differentially expressed genes shared between medium, low or no glyphosate treatment between pre-treatment and three weeks post-treatment. P-value cut-off was (<0.01).

Experiment 2: Epigenome Analysis

- Native methylation status follows the expected pattern of heavily methylated heterochromatin and demethylated euchromatic arms (Fig. 3)
- Most of the DMLs were demethylation, representing 94% of the total untreated, nearly 96% in the low treatment, and only 79% in the medium treatment (Table 1)
- The medium dose of glyphosate resulted in the least amount of total DMLs (Table 1)
- There are distinct regions of the genome where methylation is heavily affected by glyphosate application (Fig. 3)

Number Differentially Methylated Sites	Untreated	Low	Medium
Demethylated	164,527	128,293	64,840
Methylated	10,078	5,614	16,744
Total	174,605	133,907	81,584
Proportion of Demethylated	94.23%	95.81%	79.48%
Proportion of Methylated	5.77%	4.19%	20.52%

Table 1. The total number of differentially methylated sites detected as increased methylation or demethylation averaged across four samples within each treatment with a p-value threshold of <0.01.

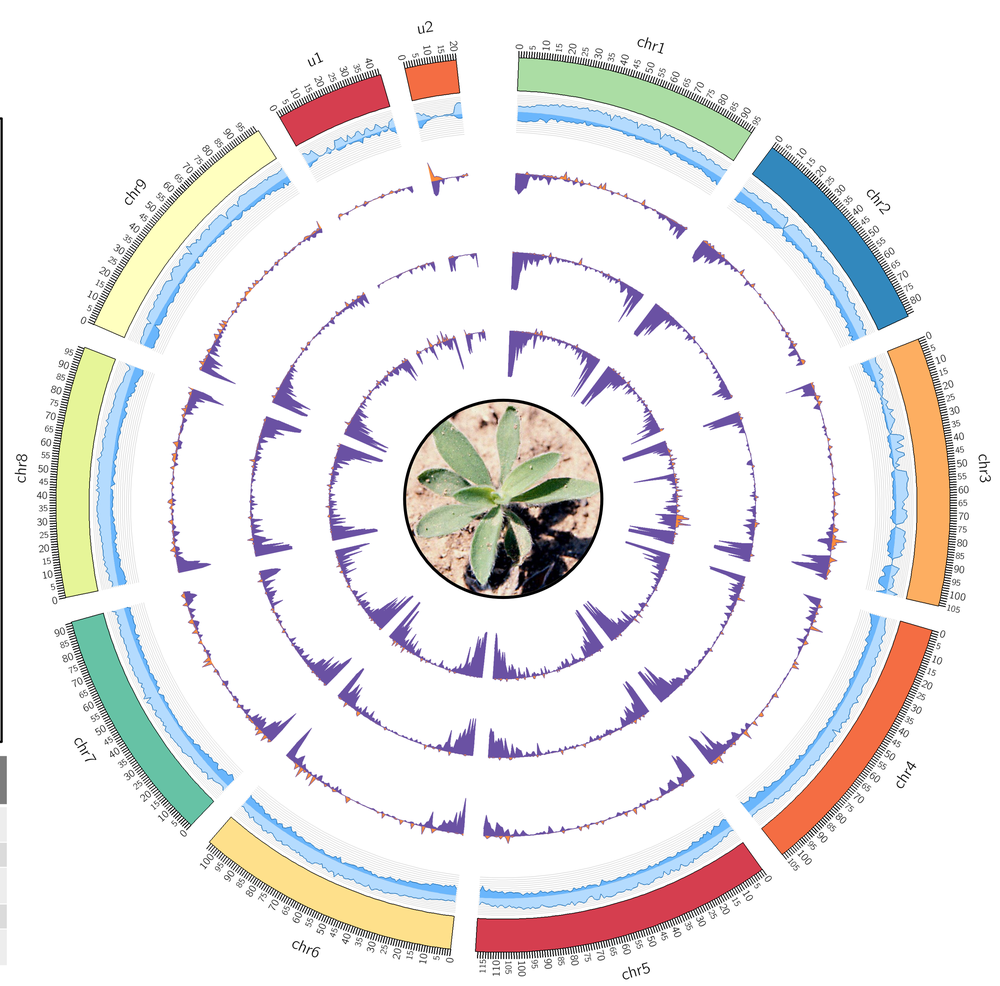

Figure 3. Genome-wide comparison of gene density, repetitive elements, and methylation changes. Light blue regions are repetitive elements and dark blue is gene density. Methylation (orange) and demethylation (purple) for medium, low and untreated are shown on a scale based on total methylated Cs.

Conclusions

- Surprisingly, untreated plants show far more differential expression then either the low or medium treated plants, indicating many normally differentially expressed genes (3,957) are arrested with even the lowest application.
- There is a significant difference in the amount of DNA methylation/demethylation following glyphosate application genome-wide with plants treated with a medium dose making less then half as many methylation changes when compared to untreated.
- Furthermore, the medium dose of glyphosate caused a significantly larger number (16,744) and proportion (20.52%) of sites to be methylated.
- We **reject our hypothesis** and instead suggest that instead of activating the genome, sublethal doses repress normal development and changes in methylation.

Future Research

Ongoing research with this data set includes:

- GO Term Enrichment Analysis of DEGs by treatment
- Intersecting transcriptomic and epigenomic data
- Identifying TEs differentially methylated and expressed
- Exploring the EPSPS region of the genome in depth

Future work will continue this experiment for a total of five generations using seed saved from the plants in this study to determine whether continued use of glyphosate induces transgenerational genomic rearrangements.

Acknowledgements

This work is supported in part by the National Science Foundation Research Traineeship Program (DGE-1828149).